REMARKS/ARGUMENTS

Drawings:

Applicant notes the acceptance by the Examiner of the drawings filed on Sept. 19, 2003.

Status of the Claims:

Claims 1 – 20 and 22 are pending. Claims 1 – 20 and 22 stand rejected.

The Examiner has maintained the rejection of the claims as being unpatentable over WO 95/33989. Applicant traverses this rejection in view of the remarks that follow.

The Examiner has rejected the previously submitted argument that the time-based aliquot embodiment of WO 95/33989 does not teach a fraction in the sense of the instant invention. The Examiner further argued that the aliquots of WO 95/33989 are fractions based on time collection, and that they are a result of a separation process.

The Examiner further rejected the applicant's argument that the selected component-based aliquot embodiment of WO 95/33989 does not teach a fraction in the sense of the instant invention in that the instant fraction contains one or more components of interest. The Examiner further argued that the applicant had failed to point to a portion of WO 95/33989 that specifies that the component be pure prior to the second separation, and argued that the applicant fails to understand that, even if the component for WO 95/33989 is pure, it constitutes a fraction as defined herein by containing "one or more components" constituting a fraction as defined in the present invention.

In response to this second point, the claims have been amended as detailed below.

The independent claims have been subject to amendment, principally to introduce two features, one of which was arguably present in the claims as they stood, but has been restated to avoid any ambiguity. Thus, it is now required that at least some of the fractions include "at least two components". It is submitted that this squarely addressing the points raised in the two preceding paragraphs by the Examiner, namely the argument that the reference in WO 95/33989 to a fraction containing a single component falls within the claims of the present invention.

The second amendment made to the claims is to make it clear that "all of the fractions" are subject to the second separation. The claims, generally had referred to "each of the fractions" being separately passed to the second separation means or subject to the second separation step. While the term "each" has a strong implication that all of the fractions are subject to the second

separation, this is now being made clear, since as argued below, it is submitted that there is no such teaching in the WO 95/33989 reference.

The passage on page 21 of the reference, previously cited by the Examiner, and at lines 15-24 of that page, refers to a technique applied to a carbohydrate mixture. It is made clear, at lines 16 of page 21 that "a selected component" is transferred to the second capillary. Indeed, in the technique taught, it is essential that a single component be transferred, since it is specified that it is transferred along with "selected enzyme reagents", with the enzyme-sample mixture being "allowed to incubate for a selected time" (line 18). This mixture is then subject to electrophoresis in the second capillary. The shift in electrophoretic mobility of the selected component between the first and second electrophoresis steps is then correlated with the known activity of the particular enzyme reagent; this necessarily requires the two electrophoresis steps to be the same, not different. At the end of this passage, at line 24, it is noted that by repeating the series of steps "each component in a carbohydrate mixture can be sequenced without any manual intervention".

Put another way, the WO reference is primarily concerned with taking one component, subjecting it to some reaction, and then, in effect, carrying out the second electrophoresis in the second capillary. In contrast, the present invention takes a fraction including two or more components, and then, as those components cannot be separated in the first separation step, subjecting each fraction to a second and different separation step, to separate those components. No intermediate reaction is required in the present invention.

Thus, the clear teaching in the WO reference, and it is submitted that this is essential for the technique described, is that each component be separated from the rest of the mixture in the first separation step. This necessarily requires that the selection of a particular component from the first step must be closely monitored, to ensure that that, desired component is indeed the one selected and transferred to the second separation.

The passage in the first full paragraph on page 20 of the WO 95/33989 reference, at lines 3-10 thereof, gives a brief outline of some alternative technique to the delay-time method, which is hard to fully comprehend from the brief description given. What is clear is that it is still intended to select a particular component for analysis, and the described technique is merely intended to be a substitute for the delay-time method. The first sentence of this paragraph is confusing. It refers to "periodically transferring an aliquot.... at predetermined, regular,

intervals". Applicants maintain that previously arguments concerning the term "aliquot", which is not defined in this WO 95/33989 reference. It seems clear that the intent is merely to provide some alternative technique, but still just to transfer a single-desired "selected sample band". Note that at page 18, lines 5 and 6, it is stated, clearly, "an important aspect of the present invention is the ability to transfer a selected transfer band..." (emphasis added). This passage goes on, further down on page 18, to make it clear that it is desired to simply transfer "a selected sample band" and not all of the fractions from a sample may be present or generated by the analysis in the first capillary.

In contrast, the present invention is concerned with a technique in which identification of a particular component or its location during the first separation step is neither desired nor is critical. In the present invention, the entire sample eluted from the first separation step is simply divided into a series of fractions. All of these fractions are then passed, sequentially, to the second separation device, in which each of the fractions is subject to separation, to identify individual components.

This technique recognizes that the location of the components within the fractions may vary greatly depending upon a number of different factors, including the nature of the material being analyzed and the particular separation techniques used. Thus, each component typically will provide some sort of peak, with the height and width of the peak varying greatly. Separation between components will, similarly, vary greatly, with some pairs of components being well separated or spaced apart during the first separation, so as to occur in distinctly different fractions, and even in some cases to have a series of blank or empty fractions between fractions containing the components of interest. In other cases, components will to a greater or lesser degree overlap one another, and it is because of such overlapping that two separation steps are used by the present invention; thus, where two components cannot be separated in the first separation step, a different, second separation technique can be used to successfully separate them. It is also conceivable that the components may not reside in a single fraction but may overlap into adjacent fractions.

Thus, the present invention as now claimed requires that the entire sample be separated into sequential fractions, and that all of these fractions be subject to the second separation. This does not require any identification of a desired component eluding from the first separation step

as in the WO reference. Accordingly, it is submitted that the claims as amended are both novel and inventive.

The Examiner has presented arguments, alleging that it would be obvious to wait until a preceding sample exited the second channel before injecting a subsequent sample. However samples may be injected in the cited WO reference, it is again submitted that this reference fails to teach analyzing all the fractions, and providing at least some of the fractions with two or more components.

A further significant aspect of the invention is the development of a true multiplexing technique that readily and simply enables a complete analysis of a sample. When carrying out a two-dimensional separation, it is almost inevitable that the first separation will need to be stopped and started, in order to enable separate fractions from the first separation to be passed into second separation means. Consequently, the present inventor has realized that this can only be achieved using certain separation techniques. In particular, techniques such as electrophoresis, which rely upon the potential gradient, are suitable for being turned on and off as desired. In contrast, may chromatography techniques, utilizing pumping and the like, are wholly unsuited to being rapidly and frequently turned on and off.

To better emphasis this aspect of the invention, the subject matter of claim 2 has been incorporated into claim 1, so that the apparatus now includes high power voltage sources across the first and second separation means. A similar amendment has been made to the main method claim 10. Claims 4, 13 and 22 have all been amended to delete reference to a variety of chromatography systems, which are not suited to rapid switching. Note that the reference to micellar electrokinetic chromatography has been retained, since this is applicable to a rapid switching scheme.

In contrast, the cited WO reference is not concerned with a true multiplexed two-dimensional analysis scheme where all fractions from a number of samples are analyzed, and moreover fails to recognize the potential advantage of using electrophoresis for such a scheme.

With respect to claims 4 and 13, the Examiner has grouped these claims in the first rejection based solely on the WO reference. However, the Examiner gives no detailed argument as to why these claims are obvious over this reference. Both these claims require selection of the separation means from a large group of different separation means. It is submitted that these different separation means are not taught in the WO reference.

Further, it is submitted that claims 2, 3, 11, 12 and 14 are all patentable both for being dependent from an allowable independent claims and for introducing further patentable features.

The Examiner further rejected claim 15 as being obvious in view of the disclosure in Moring. The Examiner relied upon the disclosure in Moring for teaching a fluorescent label, acknowledging that the WO reference fails to teach such a feature.

It is submitted that in view of the analysis techniques taught in the WO reference, there is no reason or basis in this art for considering this combination to provide the missing fluorescent label.

The Examiner further rejected claims 5-9, 16-20 and 22 under 35 USC 103(a) as being unpatentable over the WO reference further in view of Yeung et al. The Examiner acknowledged that the WO reference fails to teach a plurality of interfaces in a manifold.

The Examiner then referred to the Yeung et al. reference for teaching a system including a manifold 26 that connects a channel 90 to valve 22 which is connected to a syringe pump 19, and a variety of reservoirs via ports 53-57. The Examiner argued that this manifold is coupled to interfaces 32 which couple chromatographic columns 14 to electrophoresis capillaries 33 and outlet channels coupled to manifold 31.

The Examiner argued that it would have been obvious to one of ordinary skill to use a manifold as taught by Yeung et al. in the apparatus and method of WO reference.

This argument is respectfully traversed. Fundamentally, Yeung et al. does not describe any integral, single manifold structural element providing the features of the present invention. More particularly, the WO reference is not concerned with analyzing a number of samples, so that there is no reason or basis to combine these references as suggested.

Thus, first considering claim 7, this claim requires a manifold providing a plurality of interface regions and connected to the first and second separation means (there being a plurality of each of these separation means). In contrast, the structure in Yeung et al. is not at all clear, and at best it is schematically shown. Yeung et al. provides a so-called "cross assembly" 30 which includes a plurality of apparently separate and discrete "second junctions" 32. The second junctions 32 are, again separately, connected to join capillaries 29 and capillaries of the separation capillary array 33. These second junctions 32 are also connected to second and third manifolds 26 and 31. This general structure is discussed in the passage at column 6, lines 1-62. No detail is given here of the structure of manifolds 26, 31. It can be noted that the first junctions

13 are shown very similarly to the second junctions 32 and are clearly indicated to be connected to "intake capillaries" 3, with the implication that the various manifolds, including the first manifold 15 connected to the first junctions 13, comprise a plurality of short capillary section, i.e. not some true, integrated manifold structure.

It can further be noted that claim 7 has been amended to make it clear that the manifold is formed as a single, unitary body, a feature that is not taught in Yeung et al. Thus, even the notional combination of these two references fails to arrive at a combination as now defined in claim 7.

It can further be noted that Yeung et al. teaches an extremely elaborate valving system described as a "multiplexed freeze/thaw valve assembly (MFTV)". This is shown in detail in Figure 5. Again, such a complex valve assembly is required, where flows are required to be turned on and off. In an electrophoresis analysis scheme, or other similar analysis schemes relying upon potential fields and the like, no such valving is required. Yet, Yeung et al. fails to realize this possibility, and it noteworthy for specifically teaching the use of chromatographic columns for the first separation step.

It is further submitted that, with respect to claim 8, the structure defined by this claim is clearly nowhere taught in Yeung et al.

Claim 8 has been amended to require that the listed elements of the manifold are included in the "single, unitary body", to emphasize these characteristics of claim 8. No new matter has been added, and the Examiner is referred to page 24, lines 19-25, by way of example, where it is mentioned that the manifold 62 can be formed in plastic, glass, silicone or other suitable material.

Claim 8, as amended, thus requires the interface regions to be formed in the single, unitary body and for the manifold to include an inlet for connection to buffer reservoirs. The channel network is now defined as being within the single, unitary body and including, for each interface region, respective ports. These amendments are intended to give a clear definition of the structure essentially shown in Figure 4 of the present application.

It is submitted that, in view of the comments on Yeung et al. above, no such arrangement is anywhere taught in this reference.

With respect to claim 9, it is submitted that this claim is allowable for introducing further patentable features and for being dependent from an allowable claim.

As to claim 6, the Examiner has given no specific argument with respect to this claim, but rather has simple included it in the general rejection detailed in paragraph 23 of the Action. Claim 6 is directed to the feature of providing isoelectric focusing electrophoresis system for the first separation and sieving electrophoresis system for the second separation. These two separate and distinct separation techniques are not taught in the references, the Examiner is requested to provide a clear basis for rejection of this claim, or otherwise to withdraw the rejection. Similar comments apply to claim 16. Again, the choice of these two separation techniques facilitates the provision of a true two-dimensional multiplexed analysis scheme.

With respect to claim 17, it is submitted that the arguments above in relation to claim 1 are applicable. Similarly, it is submitted that, for claim 18, the arguments above in relation to claim 7 and 8 are applicable, and this claim 18 is also allowable for introducing the manifold concept.

As to claims 19 and 20, the Examiner made the sweeping and unsupported argument that it would have been obvious to use a known sample application device for separating components of a single cell. This argument is respectfully traversed.

With respect to claim 19, this has been made into an independent claim, to better emphasis this aspect of the invention. The application of this invention to analysis of single cells is considered important and significant. Previously, analysis of the components of a single cell, using for example a single separation technique, might have yielded information on a few of the components or compounds within the cell. With the present invention, providing two-dimensional separation, it is conceivable that hundreds of components in a cell can be characterized.

Further, the invention provides for multiplexing, i.e. the ability to analyze a large number of single cell simultaneously. This is believed to be of significance in many areas of medical research, more particularly, for example, research into cancer. It is believed that analysis of single cells will show that individual cells, e.g. from a tumor, have different characteristics, and the variation in characteristics itself may alter, as a tumor develops. It will be appreciated that investigation into such characteristics can only be achieved if one can effectively complete a comprehensive analysis of a large number of cells, and no other technique enables such an analysis to be carried out.

Accordingly, it is submitted that claim 19 and its dependent claims are allowable. In view of the amendment to claim 19, the subject matter of claim 18 has been retained, in effect, by the introduction of new, dependent claim 23. Again, no new matter has been added.

As to claim 22, it is again argued that the different separation means are not taught in the references cited by the Examiner.

The Examiner further rejected claims 1, 4-5, 7-9, 10, 13-14, 17-20 and 22 under 35 USC 103(a) over Yeung et al. in view of WO.

The Examiner argued that Yeung et al. teaches a system for performing chromatography followed by electrophoresis or other separation. The Examiner also argued that this reference teaches laser induced fluorescence, but acknowledged that Yeung et al. failed to teach sequential injection of fractions.

The Examiner argued that it would have been obvious to inject sequential fractions, etcetera. This argument is respectfully traversed. As noted above, the claims as now amended call for all of the fractions to be received by the second separation means, and for at least some of the fractions to include at least two components. Further, the claims now require that the second separation means be different from the first separation means. Such a technique is not taught in any theoretical combination of Yeung et al. and the WO reference, and it is further argued that there is in any event, no reason or basis to combine the references as suggested by the Examiner. The other claims are submitted to be allowable for introducing further patentable features and for being dependent from the independent claims, submitted to be allowable.

Again, independent claims 1 and 10 have both been amended to make it clear that the separation means require the use of a high voltage power source. In contrast, Yeung et al. teaches the provision of chromatographic columns for a first separation means, and then teaches the complex freeze/thaw valve structure to control flow. There is simply no recognition in either of the two references of the possibility of using voltage-controlled separation means, to give a true multiplexed, two-dimensional analytical technique. Again, where separation is determined by a potential field, switching each separation means on and off is simple, and enables a large number of different samples to be analyzed, or multiplexed, simultaneously.

In paragraphs 38, 39 and 40 the Examiner presented arguments and commentary on arguments previously submitted. More specifically, the Examiner argued that the WO reference contains no language requiring that the fraction is a single band and nothing more. The Examiner

is referred to the passage on page 21, lines 15-24 of the WO reference. There, it is expressly stated that "a selected component is transferred to a second capillary along with the addition of selected enzyme reagents..." The clear intention is to select just a single component, and, knowing that just a single component is present, one can then incubate this sample with the enzyme reagents. The incubating mixture is then subjected to electrophoresis in the second analysis step. It is then states, at lines 20-22 that the "shift in electrophoretic mobility of the selected component between the first and second CE dimensions is then correlated with a known activity of the particular enzyme reagent used". This is a wholly different approach from the present invention.

The Examiner referred to the sentence at page 10, lines 23-25 of the present specification (noted by the Applicant) where it is stated that each fraction "may contain one or more components". This is certainly true for the present invention. However, in the WO reference, it seems clear that the intent is that each aliquot contain just a single component, and the function of the second separation technique is not to separate two or more component that may be present in a single aliquot, but rather to carry out some different analysis step on a single component.

The Examiner noted that the WO teaches a detector 52 for detecting bands exiting the first capillary and basing the transfer of the bands to the second capillary on detection by the detector 52 (page 18 of the WO reference). The argument here is not understood. Surely, this supports the Applicant's argument that the WO reference is carefully noting the arrival of each band or component at the exit of the first capillary, so that individual and separate components can be transferred to the second capillary. In contrast, the present invention requires no such detection and separation of individual components.

The Examiner noted that the present claims do not preclude passage of aliquots containing no components. This is certainly feasible. However, what the claims do require, as amended, is that at least some of the fractions include at least two components. For some analyses, it is quite conceivable that one may have a number of components relatively close together and then a large gap, corresponding to a series of fractions, containing few or no components.

The Examiner further noted that the instant apparatus claims do not include a controller operative to perform the method. The Examiner's point here is not understood.

In paragraph 40 of the Action, the Examiner argued that Applicant had pointed to no portion of the WO reference requiring that the component be pure prior to the second separation, and the Examiner further argued that, even if the component of the WO reference is pure, it constitutes a fraction as defined wherein. Again, as argued above, it is submitted that the WO reference clearly requires just a single component to be transferred in each aliquot, and nowhere considers the transfer of a fraction containing two or more components, with the intent that these will be separated in the second separation.

Having fully and completely responded to the Office Action, Applicant submits that all of the claims are now in condition for allowance, an indication of which is solicited. If there are any outstanding issues that might be resolved by an interview or an Examiner's amendment, the Examiner is requested to call Applicant's attorney at the telephone number shown below.

To the extent necessary, a petition for an extension of time under 37 C.F.R. 1.136 is hereby made. Please charge any shortage in fees due in connection with the filing of this paper, including extension of time fees, to Deposit Account 500417 and please credit any excess fees to such deposit account.

Respectfully submitted,

MCDERMOTT WILL & EMERY LLP

#46,692

for Paul Devinsky
Registration No. 28,553

600 13th Street, N.W.
Washington, DC 20005-3096
(202) 756-8000 PD:mcm
Facsimile: (202) 756-8087
Date: October 5, 2004